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Systemic RNA interference in locusts: reverse genetics and possibilities for locust pest control

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RNA interference (RNAi) is a biological process triggered by double stranded (ds)RNA that results in sequence-dependent mRNA degradation. Because of its high specificity, this post-transcriptional gene silencing mechanism is a widely used tool for reverse genetics in several insect species. In particular, locusts possess a very robust and sensitive RNAi response that has already been exploited to investigate a diverse range of important physiological processes. These orthopteran insects constitute important model organisms in several areas of entomology, but they can also become voracious swarming pests that threaten the agricultural production in large parts of the world. In comparison to the widely applied chemical insecticides, the RNAi-technology could contribute to the development of a novel generation of insecticides, with high species-specificity. In this article, we discuss the potential of the RNAi-technology in loss of function studies in locusts, as well as to control locust populations.

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Introduction

RNA interference (RNAi) is a post-transcriptional gene-silencing mechanism triggered by long double (ds)RNA molecules [1]. These RNA duplexes are typically associated with the presence of viral infections and constitute an important virus-associated molecular pattern for antiviral immunity. In insect cells, dsRNA activates the RNA interference (RNAi) response that results in sequence specific degradation of complementary viral transcripts. Yet, by introduction of insect-specific dsRNA fragments, RNAi can also target endogenous transcripts of the insect [2]. Thanks to its specificity, RNAi has become a widely used research tool for reverse genetics. Furthermore, several recent studies have demonstrated that RNAi may also contribute to the development of novel strategies for selectively controlling agricultural pests or for curing beneficial and vector insects from viral infections [3,4]. The dsRNA is often delivered to insects by intra-abdominal injection or through feeding. In this way, the target tissue must be able to take up the dsRNA from the extracellular environment and, subsequently, induce RNAi. This biological process is referred to as systemic (sys)RNAi [5]. Once the dsRNA has entered the target cells, Dicer endonucleases will cleave it into small interfering (si)RNAs that guide the degradation of complementary transcripts by Argonaute containing RNA-induced silencing complexes (RISCs) [2]. The intracellular RNAi machinery seems to be strongly conserved among insects [6–8,9••]. Although some differences have been reported, namely a variable number of Argonaute proteins, a direct link to the RNAi efficiency remains to be demonstrated [8].

SysRNAi has been observed in many different insect species, belonging to diverse insect orders. Still, the degree of sensitivity towards RNAi varies strongly among insects, with many economically important species being refractory to systemically delivered dsRNA [7,10]. Several recent studies have illustrated that locusts are highly sensitive to intra-abdominally injected dsRNA, while naked dsRNA delivered through feeding could not induce efficient RNAi-responses [9••,11••,12••].

Locusts have served as model organisms in diverse areas of insect biology, such as endocrinology, neurophysiology, biochemistry, behaviour and ecology. Their phylogenetic position and relatively large body size, as well as the fact that they are amenable to mass rearing in captivity, are clear advantages of using locusts as research models. Furthermore, huge swarms of locusts have posed a threat to agricultural production since many centuries, as mentioned in the Bible and the Quran. They have the fascinating ability to occur in different phases, a solitarious and gregarious one, that can gradually pass into each other, resulting in obvious phenotypic differences in behaviour, colouration, morphology and physiology [13]. This biological process that is indicative of the extreme phenotypic plasticity of locusts is termed 'phase transition'. It can lead to the formation of locust hopper bands and of adult locust swarms [14]. For many years, the control of locust outbreaks was carried out by spraying millions of litres of chemical pesticides, resulting in toxic non-target effects on humans and their environment [15].

Currently, preventive and early warning strategies are also employed, involving the spraying of relatively small groups of locusts with minimal quantities of pesticides before they are able to gather and spread to agricultural areas (FAO). In this review, we discuss the importance of RNAi for reverse genetic research in locusts and the potential of RNAi in the control of locust populations.

RNAi-based reverse genetics in locusts

The best-studied locust species, *Locusta migratoria* and *Schistocerca gregaria*, show highly robust RNAi responses to intra-abdominally injected dsRNA [9••,16]. In addition, the genome of *L. migratoria* has been published [17] and an extensive transcriptome database is available for *S. gregaria* (our unpublished data), making locusts attractive organisms for 'loss of function' analyses. Since the first experiment using RNAi in locusts was published nearly ten years ago [18], RNAi has contributed to the study of many important biological processes, including ecdysis [19], digestion [20], feeding [21], reproduction [22], development [23], immunity [24••], and phase transition [25,26,27••]. In Table 1, an overview of genes investigated with RNAi in locusts is shown. In these studies, many different tissues were targetted with RNAi; such as *L. migratoria* fat body [28], hind legs [29], brain [29], thoracic ganglia [29], caeca [20] and midgut [20]; and *S. gregaria* fat body [30], brain and optic lobes [31], corpora allata [32], suboesophageal ganglion [31], thoracic ganglia [27••], malpighian tubules [19], testes [33], ovaries [9••], male accessory glands [33], trachea [9••], prothoracic glands [32] and midgut [12••]. The use of the RNAi technique has also been reported for the American locust, *S. americana* [18,23].

In addition, detailed RNAi tissue-sensitivity studies have been published. Interestingly, although locusts are highly

Table 1

Overview of genes that have been successfully silenced by using the RNAi technology. The locust species and tissues that were targeted are also indicated.

Genes	Species	Stage	Reference
Type I signal peptidase subunit	*L. migratoria*	5th instar	[54]
Methoprene-tolerant; *vitellogenin receptor*	*L. migratoria*	Adult	[34]
pyruvate dehydrogenase E1 β	*L. migratoria*	Adult	[55]
DP-N-acetylglucosamine pyrophosphorylases 1 and *2*	*L. migratoria*	5th instar	[56]
krüppel homologue 1; chitin metabolism; *vermiform*; *chitin synthase 1*; *V-ATPase subunit A* and *E*; *α-tubulin*	*L. migratoria*	4th instar	[11••]
dicer1	*L. migratoria*	Adult; 4th + 5th instar	[24••]
argonaute1	*L. migratoria*	Adult	[28]
carboxylesterases A1 and *A2*	*L. migratoria*	2th instar	[53]
glucan recognition protein 3	*L. migratoria*	Adult	[48]
V-ATPase subunit H	*L. migratoria*	Adult	[42••]
sid1; *ecdysone receptor*; *V-ATPase subunit D*	*L. migratoria*	4th instar	[16]
carnitine acetyltransferase and *palmitoyltransferase*	*L. migratoria*	4th instar	[29]
β-1,3-glucan recognition protein βGRP	*L. migratoria*	Adult	[57]
vesicular amine transferase 1; *phenylalanine hydroxylase*; *tyrosine hydrosxylase*	*L. migratoria*	4th instar	[26]
pancreatic secretory trypsin inhibitor	*L. migratoria*	5th instar	[20]
chitine synthase 1	*L. migratoria*	2th instar	[50]
hunchback	*L. migratoria*	Parental RNAi: embryo	[47,58]
protease TSP	*L. migratoria*	Adult; 4th + 5th instar	[49]
V-ATPase subunit 16; *clathrin*	*S. gregaria*	Adult	[59]
dsRNases 1, *2*, *3* and *4*	*S. gregaria*	Adult	[12••]
Short neuropeptide F	*S. gregaria*	Adult	[60]
Short neuropeptide F receptor	*S. gregaria*	Adult	[21]
Neuropeptide F	*S. gregaria*	Adult	[22,31,61]
Shade; *CYP6H1*	*S. gregaria*	5th larval	[19]
CRF diuretic hormone	*S. gregaria*	Adult	[62]
Period; *timeless*	*S. gregaria*	Adult	[33]
Alpha-tubulin; *glyceraldehyde-phosphate-dehydrogenase*; *dicer2*; *argonaute2*	*S. gregaria*	Adult	[9••]
cAMP-dependent protein kinase catalytic subunit 1 and *regulatory subunit 1*	*S. gregaria*	5th instar	[27••]
Spook; *phantom*	*S. gregaria*	5th instar	[32]
Fruitless	*S. gregaria*	Adult	[63]
Neuroparsin 1, *2*, *3* and *4*; *insulin related peptide*	*S. gregaria*	Adult	[30]
Eyes absent; *sine oculis*	*S. americana*	Adult	[23]
Vermilion	*S. americana*	Adult	[18]

responsive to RNAi in almost every tissue, a decreased sensitivity has been demonstrated to occur in the gonads [9••,34].

RNAi for locust pest control

In order to perform successful control of locust populations, it is important to consider that, by contrast to other insects, these animals present a remarkable phenotypic plasticity. On one hand, when in the solitary phase, locusts are harmless and do not constitute an agricultural threat. On the other hand, gregarious locusts can form devastating swarms that are very hard to control [14]. Therefore, the most effective targets for this task are groups of gregarizing locusts that can be detected by maintaining a close surveillance in the field. Hereupon, pesticides can contribute to control the population density and, consequently, prevent the gregarization process and thus also the formation of hopper bands, as well as migrating and devastating adult swarms. For this reason, locust populations are constantly monitored by the Food and Agriculture Organization of the United Nations (FAO), which also provide very concrete guidelines for the control of these insects [15,35]. In the course of the past decades up to the present days, the pesticides used in locust control were mainly neurotoxic chemicals such as organochlorines, organophosphates, carbamates, pyrethroids, phenylpyrazoles and chloronicotinyls (either individually of combined) [15]. Nevertheless, these pesticides present a broad spectrum of action, highly affecting other insects and, in some cases, even other classes of animals such as birds and fishes. Furthermore, they can have considerable toxicity for humans. Chemicals with a narrower spectrum of action and lower off-target toxicity (e.g. insect growth regulators) have also been used, as well as biological pesticides (e.g. the mitosporic fungi *Metarhizium*). Yet, although these last two groups of insecticides are less toxic to humans and the environment, they are very slow in action when compared to the traditional ones [15]. In addition, the development of resistance by the locusts is a notable limitation to the use of any pesticide, namely to the use of some of the referred products [15,36]. Consequently, the search for novel pesticides for locust control is fundamental and, in this context, the RNAi technology may constitute a great asset. Besides its important application in reverse genetics research, where it can help to identify novel targets for pest control, RNAi also holds great potential in contributing to insect pest control strategies. In theory, every gene can be silenced, giving RNAi the potential to affect different physiological processes and, therefore, providing a wider chance to evade resistance development. Moreover, since RNAi results in sequence-specific mRNA degradation, the selection of the appropriate mRNA target and the design of different specific dsRNA molecules could result in high species specificity, as demonstrated by Whyard and co-workers for *Drosophila* flies [37].

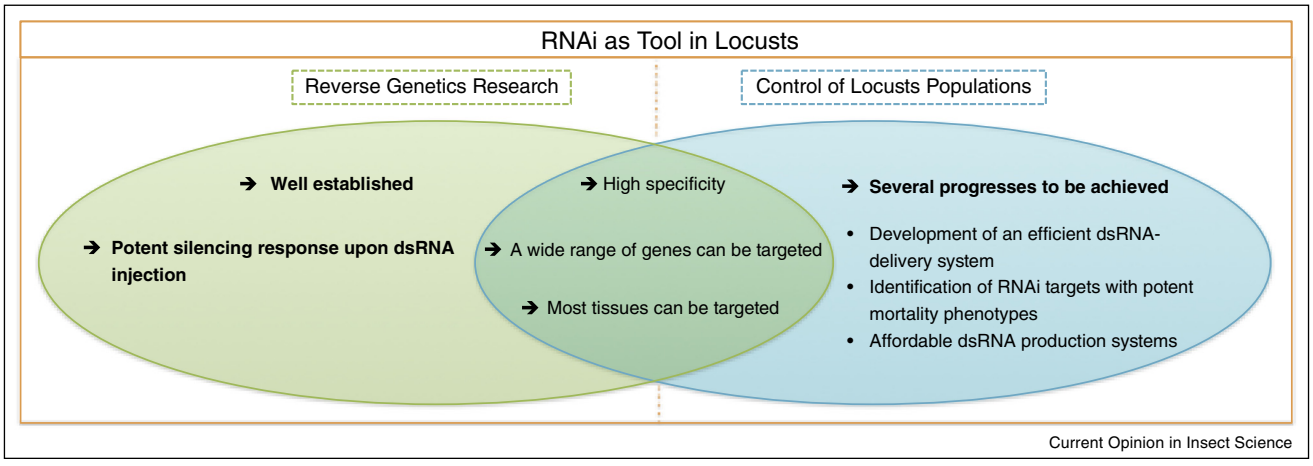
RNAi-based locust control in the field

When considering RNAi-based control of locusts, it is crucial to take into account the poorly predictable nature of the outbreaks, both spatially and temporally, as well as the locusts' ability to migrate over long distances. These facts may make the use of transgenic plants expressing dsRNA less feasible and emphasize the need to develop cost-effective methods to produce large quantities of dsRNA. Interestingly, the use of dsRNA-expressing bacteria was demonstrated to be efficient in triggering gene silencing in other insects [38••,39,40]. Although this matter has not been investigated in locusts yet, it could be a promising approach since it has already been shown to be efficient in *Acheta domesticus*, the house cricket [38••,39]. Nevertheless, it is noteworthy that, although locusts are very sensitive to RNAi upon injection of naked dsRNA in the haemocoel, these insects seem to be refractory to dsRNA delivered by feeding [11••,12••]. There are several reasons proposed as being responsible for the low efficacy of orally delivered dsRNA, such as the degradation of the dsRNA before the cellular uptake in the digestive system and the possible absence of an efficient dsRNA-uptake system in the gut [12••]. However, this is still a matter of debate and more studies are needed to clarify this. Fortunately, in insects equally irresponsive to feeding of dsRNA, successes have been obtained with dsRNA-delivery systems. Some examples are the feeding of liposome-coated dsRNA or of polymer containing (chitosan/dsRNA) nanoparticles [37,41]. Therefore, although no dsRNA-delivery system by feeding is available for locusts at the moment, reports from other insects positively encourage the development of such an oral application method.

Targets for RNAi-based locust control

Up to now, the shortest time-frame in which RNAi-induced mortality could be triggered in locusts was 7–14 days, upon the injection of dsRNA. This was achieved by performing the knockdown of genes involved in important general functions, such as *V-ATPaseH* and *V-ATPaseD*, or of genes involved in basic cellular functions, such as *alpha-tubilin 1a* [9••,16,42••]. Interestingly, in other insect species, the delivery of dsRNA by feeding led to strong post-transcriptional silencing in the gut and consequent mortality, which highlights the potential of targeting vital genes in this tissue [37,43,44]. Although the mentioned mortality-inducing time frame is considered slow, when compared to some of the traditional insecticides (in some cases, able to induce mortality in periods starting from eight hours) [15], several strategies may be followed in order to optimize this timing, as for instance the combined use of several targets or the concerted use of RNAi with other insecticides. Moreover, more rapid mortality-inducing targets must be identified, as well as possible strategies to impair the gregarization process.

Figure 1



RNAi as a tool in locusts. Overview of the important parameters for the use of RNAi for reverse genetics in locusts (blue), of the important factors that should be investigated for the development of RNAi-based control of locust pest populations in the field (green) and important advantages for both applications (overlap of both colours).

Several other interesting targets include genes involved in the reproductive cycle. Although the gonads were demonstrated to be less sensitive to RNAi in locusts, it has been demonstrated that knocking down genes mainly expressed in these tissues can still strongly affect reproduction in *S. gregaria* (e.g. the circadian clock genes *period* and *timeless*) [45,46]. In addition, several genes involved in the reproductive cycle are highly expressed in other tissues where the RNAi-response is very robust (e.g. *fruitless* and *neuropeptide-F*) [22,45]. Interestingly, the triggering of parental RNAi for genes involved in embryonic development (e.g. *hunchback*) was successful in compromising the progeny and, therefore, also constitutes a promising approach [47]. Furthermore, in locusts, it has been demonstrated that RNAi can be employed to impair ecdysis (e.g. *ecdysone-receptor*, *dicer-1* and *chitin-synthase-1*) [16,48–50]. An additional attention-grabbing approach is to consider the locusts' phase transition process, for instance by reducing the gregarious behaviour during crowding (e.g. silencing of *cAMP dependent protein kinase catalytic subunit 1*, which was demonstrated to be linked to a reduced susceptibility for gregarious behaviour in the gregarization process) [27••]. In this case, instead of causing mortality, locusts could be forced to remain in their naturally occurring harmless solitary phase.

Finally, results of the use of RNAi in combination with chemical or biological insecticides in locusts seem very promising. In the first place, the knockdown of genes controlling immunity together with the use of entomopathogenic agents may reveal to be very efficient in pest control. An interesting example is the biological control of locusts with the fungus, *Metarhizium anisopliae* (var. *acridum*), a widely used biological control agent. It has been demonstrated that silencing the glucan recognition protein 3 significantly shortened the life span of gregarious locusts upon infection with the fungal agent. Similar strategies could be designed for other locust pathogens, such as the microsporidian *Paranosema locustae* [24••,51]. Still within this scope, a second very interesting strategy may reside in the combination of traditional pesticides with the knockdown of genes involved in insecticide detoxification. In this context, the silencing of several carboxylesterase genes of the migratory locust could successfully increase the efficacy of several traditional pesticides [52,53].

Conclusions and future perspectives

Throughout this review we summarized the (potential) applications of the RNAi technology in locust research and in the control of locust populations in the field. Although this technique is already successfully used in reverse genetics research in these animals, its application in locust pest control is still dependent on the optimization of several factors such as the development of a sustainable dsRNA production method, the availability of an effective system for delivery in the field and the identification of new target genes to decrease the population density (Figure 1).

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